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What is claimed is:

Claims

1. A method of controlling cancer suppression in a mammal having a cancer suppressing gene, comprising the steps of:

making a substantially duplicated genetic material corresponding to the genetic material of said gene, the substantially duplicated material selected from the group consisting of a cloned cancer suppressing gene, a modified or defective cancer suppressing gene, homologues thereof, fragments thereof, and mixtures thereof; and

interchanging said duplicated genetic material and the cancer suppressing gene of the mammal.

- 2. A method of claim 1, wherein before said making a substantially duplicated genetic material, determining the chromosomal location of said cancer suppressing gene of the mammal.
- 3. A method of claim 1, wherein after said making a substantially duplicated genetic material, detecting the presence or absence of an inactive cancer suppressing gene of a tissue sample of the mammal to determine whether or not the tissue sample cancer suppressing gene is defective or absent.

- 4. A method of claim 3, wherein in response to a determination that the tissue sample cancer suppressing gene is either defective or absent, replacing a cancer suppressing gene of the mammal with its clone.
- 5. A method of claim 3, wherein the determination of whether or not the tissue sample cancer suppressing gene is defective or absent is accomplished by measuring the amount of protein product of said cancer suppressing gene, of the tissue sample, bound by an antibody specific for said protein.
- 6. A method of claim 5, wherein the determination of whether or not the tissue sample cancer suppressing gene is defective or absent is accomplished by:
- (a) labeling said tissue sample with radioactive isotope;
 - (b) lysing the labeled tissue;
- (c) reacting the protein product of said cancer suppressing gene with an antibody specific for said protein thereby forming a protein/antibody immunocomplex;
- (d) autoradiographing the immunocomplex
 obtained in step (c); and
- (e) determining the presence or absence ofthe protein product by comparing the autoradiogram of step(d) with the autoradiogram of the standard protein product.

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- 7. The method of claim 5, wherein the determination of whether or not the tissue sample cancer suppressing gene is defective or absent is accomplished by enzyme immunoassay techniques.
- 8. The method of claim 5, wherein the determination of whether or not the tissue sample cancer suppressing gene is defective or absent is accomplished by immunocytochemistry methods.
- 9. The method of claim 5, wherein the cancer suppressing gene is the RB gene and the protein product is ppRB110.
- 10. The method of claim 1, wherein said cancer suppressing gene is replaced with substantially duplicated material selected from the group consisting of said cloned cancer suppressing gene, homologues thereof, fragments thereof, and mixtures thereof, for therapeutic purposes.
- 11. The method of claim 1, wherein said cancer suppressing gene is replaced with substantially duplicated material selected from the group consisting of said defective cancer suppressing gene, homologues thereof, fragments thereof, and mixtures thereof, for facilitating the testing of the carcinogenicity of environmental influences.



- 12. The method of claim 2, wherein the location of said cancer suppressing gene is determined by chromosome walking.
- 13. The method of claim 2, wherein the location of said cancer suppressing gene is determined through organic markers.
 - 14. A method of claim 2, wherein:

said chromosomal location of said cancer suppressing gene is determined by testing genes of a chromosome for phenotypic expression;

determining one of the genes of said chromosome to be a marker gene; and

using chromosomal walking techniques to locate a cancer suppressing gene.

- 15. An animal genetically altered so as to have the allele of at least one cancer suppressing gene selected from the group consisting of a defective allele, a homologue thereof, a fragment thereof, and a mixture thereof.
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 16. An animal of claim 15, wherein said defective allele is selected from the group consisting of defective alleles of RB genes, breast cancer suppressing genes, Wilm's tumor suppressing genes, Beckwith-Wiedemann syndrome suppressing genes, bladder transitional cell carcinoma

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suppressing genes, neuroblastoma suppressing genes, small cell lung carcinoma suppressing genes, renal cell carcinoma suppressing genes, acoustic neuroma suppressing genes, colorectal carcinoma suppressing genes, homolgues thereof, fragments thereof, and mixtures thereof.

- 17. An animal of claim 15, wherein said allele contains a DNA fragment having at least one defective nucleotide sequence.
- 18. An animal of claim 15, wherein said defective allele contains a DNA fragment having at least one defective RB nucleotide sequence.
- 19. The animal of claim 15, wherein said animal is a mouse.
- 20. A method for determining the carcinogenicity of suspected environmental influences, using the animal of claim 14, comprising the steps of:

exposing said animal to a suspected environmental influence;

observing the animal for the phenotypic expression of cancer; and

determining carcinogenicity of the suspected environmental influence in response to observing a phenotypic expression of cancer in the animal.

- 21. A method of claim 20, wherein said exposing includes exposing to a source of radiation.
- 22. A method of claim 20, wherein said exposing includes exposing to tobacco combustion products.
- 23. A method of claim 20, wherein said exposing includes exposing to food additives.
- 24. A method of claim 20, wherein said exposing includes exposing to artificial substances.
- 25. A method of claim 20, wherein said observing includes examining the animal for tumor development.
- 26. A method of claim 25, wherein in response to the formation of a tumor in the animal, analyzing the tumor for the presence of cancer cells.
- 27. A method of making the animal of claim 15, comprising:

using at least one allele of an animal cancer suppressing gene selected from the group consisting of a defective allele, a homologue thereof, a fragment thereof, and a mixture thereof:

mutating at least one animal cell with said allele to form a mutated cell:

introducing said mutated cell into an animal
blastocyst;

permitting growth of the blastocyst for a given period of time sufficient to incorporate said allele into its cells; repressing genetic recombinations within said cells; transferring the blastocyst containing said allele into the uterus of a pseudo pregnant animal for giving birth subsequently to an animal bearing said allele;

breeding said animal to reproduce additional animals; and

selecting the animal of claim 14 from said additional animals by determining the presence therein of the said allele.

- 28. A method of claim 27, wherein before introducing said allele, removing said blastocyst from a super ovulated animal, and wherein said blastocyst is comprised of undifferentiated cells.
- 29. A method of claim 27, wherein said introducing is performed in vitro.
- 30. A pharmaceutical composition wherein the active ingredient is selected from the group consisting of a naturally occurring intact cancer suppressing gene, a cloned intact cancer suppressing gene, fragments thereof, homolgues thereof and mixtures thereof.
- 31. A pharmaceutical composition of claim 30, wherein said naturally occurring and cloned cancer

suppressing gene is selected from the group consisting of RB genes, breast cancer suppressing genes, Wilm's tumor suppressing genes, Beckwith-Wiedemann syndrome suppressing genes, bladder transitional cell carcinoma suppressing genes, neuroblastoma suppressing genes, small cell lung carcinoma suppressing genes, renal cell carcinoma suppressing genes, acoustic neuroma suppressing genes, colorectal carcinoma suppressing genes, homolgues thereof, fragments thereof, and mixtures thereof.

- 32. A pharmaceutical composition of claim 30, wherein the active ingredient is selected from the group consisting of RB cDNA, modified RB cDNA fragment, clones thereof, homolgues thereof and mixtures thereof.
- 33. A pharmaceutical composition of claim 31, wherein the active ingredient for each of said gene is selected from the group consisting od cDNA of said gene, fragments of said cDNA, homologues thereof and mixtures thereof.
- 34. A pharmaceutical composition of claim 32,
 20 wherein the cancer suppressing gene is isolated from human chromosome 13 region 13q14.

35. A pharmaceutical composition of claim 31, wherein the cancer suppressing gene and its clone each has the following nucleotide sequence:

CAGGACAGEGGCEEGGAGGACCTGCCTCCTCGTCAGCCTTGAGTTTGAAGAACCAGAACCTGATTTTACTGCATTATGTCAGAAATTAAGATACCACATCATGTCAGAACGAGCT A D B A P E D L P L V A L E P E E T E E P D P T A L C A K L K I P D A V R E B A (74) A TENTELLIANCE GROSSING TO A TOTAL SECTION ASSESSMENT OF THE PROPERTY OF THE P TITELOCHIELEVNKIINLLKEIDTETKVDHANGALLKE (1949 OVLIALISKLERICELITLIAPSSISTE.IHSALV.LKYS.(196) THE THE PARTY OF THE PROPERTY 1 1 1 L L A R G E V L G M E D D L V L S F G L M L C V L D Y F 1 K L S P P N L (234) TRIAVIPING SPRIPRRGONRSARIAKOLENDIRE emeliciciellymemewielwiatwiwingereelemwyleiliviliemwyllitviveclilitalewileicicliedweligiweveleiwigereile EAFCEERECHIPEAKRALLE BARRELE A COMPANICATION OF CONTRACTOR A COMPANICATION OF CONTRACTOR AS CONTRACT CHANGE STREET AND ASSESSED FOR THE STREET OF THE STREET ST ERLSKRYEELYLKHKDLDARLYLDHOKTLQTDSIDSFET@ (334) RTPREBELDEEVH: VIPPHIPVRTVNNTIQGLNNILNSAS B (3PA) CANCELLEVARIANTICLEVILLESTATICATES CONTRACTOR CONTRACTO BENLISTINNETVNPRESILKRVKDIETIJKEKIA-KAY (SM) SENCY DESCRIPTION OF THE PROPERTY OF THE PROPE 6:6 CYELGSORTELGYRLYYRYHESKLESERLS: 0:8 F S (476) AMETICIGNATORCANCATITITEATATETETTTATTOSCOTOCOCTOTTORGETTETATTOSCCACATATASCAGAMETACATCTCAGAATCTTGATTCTGCAACAGATTTGTCTTC 1640 E - L H D H I F H H S L L A C A L E Y Y H A I Y S R S I S G H L D S G I D L S F (316) CENTERNITCIENTETECTTANTITANGECTTTENTTITACAMETEATERMETITATEMACACAMETEATERMETITATEMACACAMETEATAMACATTANACATTA VILHVLHLKAFDFYKVIESFIKAEGHLTRENIKHLE-REE (354) EFLEUTICVIENTIECCIA SCYLBOCICI CYGYLLCYCCIALIVI LEVICLIAVI LYVICTVI CONTRONOCONCERNING CONCLEYLOCCIA CONTROL CONTROL AND CONTR I H E S L A W L S D S P L F D L I K B S K D R E S P T CETETECROMATAMICACACTOCAGATATGIATCTTTCTCCTGTAAGATCTCCAMGAAAAAGGTTCAACTACGCGTBTAAATTCTACTGCAAATGCAGAGAACACAACCACCTCTCA 2040 L Q H H T A A D H Y L S P Y R S P K K G S T T R Y H S T A H A E T Q A T S (434) O T O E P L E T B L S L F Y K K Y Y R L. A Y L R L H T L G E R L L S E H P (676) ENVINONMENTALECTICAGE CECTALITICE PORTICE CONTRACTOR LA CONTRACTOR DE LA C LERIIVILIANTE ANTE LARDRILLE DEINNESNY 0-1 EKY (716) WATER LY TOTAL SET OF THE STATE RHIDLEFEILVTATED LPHAVGETFERVLIKEEETD-8 1 1 V (734) JÉTETET IVE GIVILLET GENERAL CONTRACTOR DE LE CONTRACTOR DE LE CONTRACTOR DE LE CONTRACTOR DE LA CONTRACTOR LRIPGEHITIEPLKEPYKISEGLPIPIKK vilediavievileresentillarentilecranivivvieveviadivialiverecarecalgiscientimanalaciantemericerecelectivicerelentivi STREER SORING HVCH SDRVLKRSAESEK CINCOCLITICATIVITETICATE CONTRACTOR CONTRACT EAD G S K H L P G E S K P G G K L A E M T S MANIGATIONIAGE TEAMERAGE MANIGAGE TEAGER TEAGER TEAGER TEAGER TEAGER TEAGER TEAGER TEAGER TEAGER TO 4777 (728)

- 36. A pharmaceutical composition of claim 32, wherein said RB cDNA fragment is selected from the group consisting of RB-1, RB-2, RB-5, y79RB and mixtures thereof.
- 37. A pharmaceutical composition of claim 32, wherein a resulting mRNA transcript of said RB cDNA fragment has 4.6 kb.
- 38. A pharmaceutical composition of claim 37, wherein the cloned genomic DNA has at least 27 exons.

39. A pharmaceutical comp sition of claim 30, wherein the cloned RB cDNA transcribes into mRNA which translates in protein having an amino acid sequence comprising:

HDVOLATHYHILLUTTPHYHOLOD PLSKFKARELSGFHAALTKQGSST PRBH B G V C Z G Y B H L H A K L I R H Z G B KIVIKELHIEFOHKLDEFILIKEK TVORLVERRIEREREVERY PROIBLIDLVHYLFDYBHTTIQDE RLVBRQHBKHCKLDBLLTAHBKGB KELVTHOVHVTLAFPSSLYIPISK TEGHOESKKIVOCYLPLOKLLIKK <u> VECKEDPHDPHVAKFVFHDQKQH</u> ZAHDBHRTHHYBBZRTYHTLLLQ EVLRYSDOSHFITLOTLODYRXR ERDLLPTLTSAQDEPQSISKIPH DESLAPROSAKHLRLASCIFLLQ **QHPYWLESROOKPHPASKFBILK** TLIBLHPDVRSYADBARTTGVQ SYTLORVLPPRLVDRYELYSHL TIPITTY DHKLLLLBKYPAPVP DKKQFGFFRLVAISKRRQPSDK S OHYVE TATELY SIGNEHBY CO EKLLIRKRPSYVSKKVLATKH B PKLTSRHLVIRHT QKYHVRISS FEEPOOILTKHTKKGLDEPERF LGTBLBHDHKHRGETLLKBPKK CIBICANKTOLSHORRDRPTRL QCTSVRSTILKTLPVLQVIPSA KIKTLILLQUBBTTHHILPTAE TEVEDAD OPE ORDSTHIKEN KIDIYKLTLIBHBHTLHKIHGT PAABILTSHKRDIEHESERPHT DVHAXESIIELSXSARHESRPR HDSLLHHDLKSCHAELYYPSPT IAHHPOVDHFELHLACOBPT88

Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Gly; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; H, Het; H, Asn; P, Pro; Q, Gln; R, Arq; S, Ser; T, Thr; V, Val; H, Trp; and Y, Tyr.

40. A DNA nucleotide sequence comprising:

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MPPKTPRKTIAATAAAAAIE (PPAPPPPEEBPE (34)
  9 9 8 9 E D L P L V R L E F E E T E E P D F T A L C Q K L K 1 P D H V R E R A (74)
  TOUTIANCE I GOVERNMENT I LE E A B & A B & A F & B & A I & B K K K E F A B I C I & I V V A A B F D E E 8 (119)
  TICACITITACTOAGCTACAGAAAAACATAGAAATCAGTGTCCATAAATTGTTTAACTTACTAAAAGAAATTGATAGCAGTACCAAAGTTGATAATGCTATGTCAGGACTGTTGAGAAG
       T F T E L O K H T E I S V H K F F H L L K E I D T S T K V D H A H S R L L K K (154)
  Y D Y L J A L J & K L E R T C E L I Y L I Q P & 8 & 1 & T E .1 H & A L Y, L K Y S (196)
1 1 FLLAR REVLANED DLY 1 8 FOLM LC
                                                                                                              Y 7 1 K L B P P H L (254)
ETCAMBANCATATAMACABETBYTATACCEATTANTGOTTCACCTCGAACACCCCAGGCCGAGGTCAGAACAGGAGTGCACGGGATAGCAAACAACTAGAAAATGATACAAGAATTATT
   LEEPYKTAVIPINGSPATPRAGGNRSARIARGLENDTRII (274)
CAMPITETETSTAMBAACATGAATGTAATATAGATGAGGTGAAAATGTTTATTTCAAAAATTTTATACCTTTTATGAATTCTCTTGGACTTGTAACATCTAATGGACTTCCAAAAGTT 1000
       (314)
  BALLATETTIETAMEGATACGATACGAACAATTATETTAMAATAAAGATETAGATGCAAGATTATTTTTGGATCATGATAAAAGTGTTCAGACTGATTCTATAGACAGTTTTGAACAGTTTTGAACAGT
ENLSERYEEIYLKHKDLDARLFLDRDKTLGTDSIDGFETG (354)
WARRECT COMMAND FANCET TOAT COARDANG TOTAL TOCCT CCACACACT COAD TAGORIC TOTTAT CAACACTAT CCAACAAT TAAT CAT CAT TAAT CAACACT TAGORIC TOTAL TAGORIC TAT CAACACTAT CAACACT TAAT CAT CAACACT TAGORIC TAT CAACACT TAGORIC T
ERTPRESELDEEVNYIPPHTPVRTVNRTIGGLNRILHSASO (3M)
B B E H F I E A L H H C I A H L K E E I
                                                                             LKRYKDIGYIJKEKI
                                                                                                                                   A-E A Y (434)
TECHELEGGETTETETEGAAATTEGATCACAGCGATACAAACTTGGAGTTCGCTTGTATTACCCABTAATGGAATCCATGCTTAAATCAGAAGAAGAACGATTATCCATTCAAAATTTTAGC 1540
   SOCYEISSERYKLGYRLYYRYNESHLKSEERALSSOHFS (474)
AMETICIANATALCALCATITICATATATETETTATIGGCGTGCGCTCTTGAGGTTGTAATGGCCACATATAGCAGAAGTACATCTCAGAATCTTGATTGTGGAACAGATTTGTGTTTC 1400
  R L L H D H I F H H S L L A C A L E V V H A T Y S R S
 PUILHVEHEKAFDFYKVIESFIKAEGHLTREHIKHLERCE (554)
 LIDLIKOCKO
 VRSPKKOSTTRVNSTANAETGATS (634)
 TRLATERENTL CERLLSERP (674)
 CANTIAGALEATATEATETEGRACCETTTTCCAGCACACCETGCAGAATGAGTATGAACTCATGAAGACAGCCATTTGGACCAAATTATGATGTGTCCATGTATGCCATATGCAAAGTG
  ELENII VILI GREYELKRORNLOGINN CSNY GICKY (714)
 MONTATARDACCITAMATICAMATCATTGTAACAGCATACAAGGATCTTCCTCATGCTGTTCAGGAGACATTCAACGTGTTTTGATCAAGGAGGAGGAGGAGTATCATTATAGAA 8400
  RHIDLEPEIVTAYEDLPHAVGETFERVLIKEREVOSIIV (754)
 TICTATALCTCOGICTICATOCAGAGACTGAAAACAATATTTTOCAGTATGCTTCCACCAGGCCCCCTACCTTGTCACCAATACCTCACATTCCTCGAAGCCCTTACAAGTTCCTAGT 2220
         H S Y F H G R L R T H I L G Y A S T R P F L S P I P H I P R S P Y R F P S C794)
 ICTECELLYCERYLLECTERMENTETTLY TALENCE CLENYONGLECTTY WAY LLICTON OF LELECTTY CONTRACTOR TO THE LEGISLECT PROPERTY OF THE PROPE
                                                                      KISEGLPTPTKMTPRBRILYS (ASA)
 EXPOKINONY
                                                                  CHSDRYLKRSAESHPPKPLKK (674)
 CINCECLILEVIVILETVOTICYOTICATECTURE EGITELTVICCICCETECTOLICETTALILEVECTORITYCIOGETETTVICATECTURE SE
                                                                                               LAEM
 MANIGUATQUIAGEATGQUIAGEATGAGGAAGAAGGAATGAGGATCTCAGGACCTTGGTGGACACCTGTGTACACCTCTGGATTCATTGTCTCTCACAGATGTGACTGTAT... 10 4737
                                                                                                                                                 (724)
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41. A method of therapeutically treating inactive, mutative or absent cancer suppressing genes comprising:

treating said inactive, mutative or absent cancer suppressing genes with at least a portion of intact cancer suppressing genes.

- 42. A method of claim 41, wherein said cancer suppressing genes are each a substance selected from the groups consisting of RB genes, breast cancer suppressing genes, Wilm's tumor suppressing genes, Beckwith-Wiedemann syndrome suppressing genes, bladder transitional cell carcinoma suppressing genes, neuroblastoma suppressing genes, small cell lung carcinoma suppressing genes, renal cell carcinoma suppressing genes, acoustic neuroma suppressing genes, colorectal carcinoma suppressing genes, and mixtures thereof.
- 43. A method of claim 41, wherein said treating includes:

treating said inactive, mutative or absent cancer suppressing gene with a substance selected from the group consisting of an RB gene, a portion of said gene, or a mixture thereof.

44. A method of claim 43, wherein said portion is selected from the group consisting of RB cDNA, RB cDNA fragment, homologues thereof and mixtures thereof.

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- 45. The method of claim 41, wherein the intact cancer suppressing gene, or portion thereof, is delivered to the site of a tumor by means of a retrovirus.
- 46. A method of claim 41, wherein the intact cancer suppressing gene, or a portion thereof, is delivered to the site of a tumor by a liposome.
- 47. A method of claim 41, wherein the location of said cancer suppressing gene is determined by utilizing a genetic marker.